EVALUATION OF VARIOUS METHODS IN THE IDENTIFICATION OF STRAIN HOMOLOGY AMONG PSEUDOMONAS ISOLATES

DISSERTATION SUBMITTED FOR THE DEGREE OF

M.D., BRANCH – IV

(MICROBIOLOGY)

MARCH - 2008

THE TAMILNADU

DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI, TAMILNADU

BONAFIDE CERTIFICATE

This is to certify that this dissertation entitled **"EVALUATION OF VARIOUS METHODS IN THE IDENTIFICATION OF STRAIN HOMOLOGY AMONG PSEUDOMONAS ISOLATES"** is a bonafide record work done by **Dr. N. RAM MURUGAN** under my direct supervision and guidance, submitted to the Tamil Nadu Dr. M.G.R. Medical University in partial fulfillment of University regulation for MD, Branch IV –Microbiology.

DR.A. UMA, M.D.,

Director Incharge,
Institute of Microbiology,
Madurai Medical College
Madurai

DECLARATION

I **Dr. N. RAM MURUGAN** solemnly declare that this dissertation titled **"EVALUATION OF VARIOUS METHODS IN THE IDENTIFICATION OF STRAIN HOMOLOGY AMONG PSEUDOMONAS ISOLATES"** has been done by me. I also declare that this bonafide work or a part of this work was not submitted by me or any other for any award, degree, diploma to any other University board either in India or abroad.

This is submitted to The Tamilnadu Dr. M. G. R. Medical University, Chennai in partial fulfillment of the rules and regulation for the award of Doctor of Medicine degree Branch –IV (Microbiology) to be held in March 2008.

Place : Madurai **Dr. N. RAM MURUGAN**

Date :

ACKNOWLEDGEMENT

I wish to express my sincere thanks to our Dean, **Dr. V. Raji. M.D.,** Madurai Medical College, Madurai for having allowed me to conduct this study.

I am extremely grateful to our Director incharge **Dr. A. Uma M.D** Institute of Microbiology, Madurai Medical College, Madurai for her constant support, invaluable suggestions and encouragement throughout my study.

I owe special thanks to **Dr. Jhansi Charles,** M.D., Additional Professor, Institute of Microbiology, Madurai Medical College for her motivation and immense help in completing the study.

I am extremely grateful **Dr. P.A.T. Jegadeeswari,** M.D., **and Dr.RVibushan,** M.D., Additional Professors, Institute of Microbiology, Madurai Medical College and **Dr.S .Chandrasekar,** MSc.PhD for their guidance and encouragement.

I express my sincere thanks to Assistant Professors, **Dr. Shanthi**, M.D., **Dr. Radha Kumari,** M.D., **Dr. Mythili,** M.D., **Dr. Ganesan,** M.D.,and **Dr. Suriya Kumar,** M.D., for their valuable suggestions given to me.

I express my sincere thanks to **Thiru.R. Paramasivan, M.Sc., D.M.V., Thiru. K.J. Dhanajayan, M.Sc.,** for providing opportunity and guiding me during the study of RAPD typing in VRME laboratory, Madurai.

I would like to thank my colleagues **Dr.Lavanya, Dr.Sudha, Dr.Vazhavanthal, Dr. Anuradha and Dr. Preethi** for their encouragement.

I also thank the technical staffs for their help rendered during the study.

I also like to thank **Mr.Kumar and R. Selvaprakash, Sri Balaji Computers** for their patient co-operation in computerizing the study.

I would like to thank my family – **Dr. S.Sumathy, R. HariBaskar** for being a constant source of encouragement.

Last but not the least I would like to express my sincere gratitude to the Lord Almighty.

CONTENTS

INTRODUCTION

Pseudomonas aeruginosa (P.aeruginosa) is a ubiquitous organism that is often found in environment. It is a Gram-negative bacillus, motile, straight (or) slightly curved measuring 0.5 – 8 micrometer by 1.5 to 3.0 micrometer, non-spore forming, and noncapsulated & obligate aerobes. Pseudomonas putida can appear elongated. Organism from old culture may appear pleomorphic Gram negative. Mucoid strains are seen as long filaments surrounded by darker pink staining material (Alginate). Gram negative bacilli that are showing intra cellular presence in polymorphonuclear leucocytes are clinically important. Flagellar staining reveals single polar flagella. It grows at wide temperature range between 6-42^{o}C .

Most pseudomonas species can reside in a wide variety of environmental niches. Since their preferred temperature requirements are between 4 and 36^{o}C, Pseudomonas are found in processed foods, in

hands of health professional and they colonise animals and human. They can spread among individuals.

Ear infection and an irritating folliculitis may be acquired from swimming pools, hot tubs. Community associated P. aeruginosa infections may occur as consequent to exposure to the organism in hot tubs, whirlpools, and swimming pools. Keratitis due to P.aerugionsa may occur in individuals who store contact lenses in tap water and or contaminated contact lens solutions. Intra venous drug users may develop endocarditis and osteomyelitis as a result of injection of contaminated injectables. P. aeruginosa is also of nosocomial significance The organism has been isolated from a variety of solutions including soaps, ointments, irrigation and dialysis fluids, eye drops and disinfectants as well as from fomites such as shower heads, respiratory therapy unit, sinks, baths etc. Populations at risk for significant morbidity and mortality include intubated patients in intensive care, patients on chronic ambulatory peritoneal dialysis, and burns patients.

Pseudomonas Species are associated with a number of infections. They produce infections of wound and burns giving rise to blue green pus. P. aeruginosa can cause meningitis usually following trauma (or) surgery. Corneal infections result from contaminated contact

lenses (or) injury, contaminated eye drops, and ointments. Pseudomonas causes endocarditis through valve replacement.

People who spend long periods for swimming are at risk for external ear infections (Swimmer's ear). Malignant external otitis is a virulent form of disease seen primarily in diabetes and elderly patients. P. aeruginosa is the leading cause of chronic otitis media.

Folliculltis is a common infection caused by Pseudomonas species resulting from immersion in contaminated water (e.g. hot tubs, whirlpools, and swimming pools). Thermal burns of skin inhibit an essential component of body defence against infection; the physical barrier of intact skin .The resulting damaged tissue is a rich culture medium and is a great risk for colonization and infection by Pseudomonas. Such infections have been the leading cause of morbidity and mortality. Infection of burn wound with Gram negative bacteria typically occur one week after injury .The moist surface of the burnt skin and lack of neutrophilic response to tissue invasion predispose patients to such infections. Infections of the urinary tract are seen primarily in patients with long-term indwelling catheter. The lungs of children with cystic fibrosis are particularly susceptible to P. aeruginosa.

Use of ventilator which may introduce the organism to lower airways produces lung abscess and pneumonia .The mortality rate is 70%.

Epidemiological investigations of P. aeruginosa infection have different typing methods as follows

1 Biotyping

2. Antiobiotic Sensitivity Typing

3. Serotyping

4. Bacteriophage typing

5. Bacteriocin Typing

6. Modified Diene's mutual Inhibition Test

7. Random Amplified Polymorphic DNA Study

The Bacteriophage and Bacteriocin typing are outdated because cross-reactions are more common.

The Diene's mutual inhibition test has been used as an Epidemiological tool to characterize Pseudomonas aeruginosa like Proteus mirabilis. The test was simple to perform and cheap and may have utility in initial screening of P.aeruginosa isolates in suspected common source epidemic.

Genomic finger printing methods are now regarded as the most accurate methods for the typing of microorganisms for epidemiological

purposes. These methods include pulse field gel electrophoresis, Ribotyping, and Polymerase chain reaction based finger printing (RAPD – PCR).

In the present study, P.aeruginosa was isolated from heterogenous clinical samples from patients attending Govt Rajaji Hospital, Madurai and were subjected to various tests to identify strain similarity. Further they were screened for similar homology using phenotypic test, Diene's mutual Inhibition test, and genotypic test RAPD – PCR.

AIM AND OBJECTIVES

This study on the **'EVALUATION OF VARIOUS METHODS IN THE IDENTIFICATION OF STRAIN HOMOLOGY AMONG PSEUDOMONAS ISOLATES'** was conducted on 183 samples collected from various infections of Govt Rajaji Hospital with the following aims:

1. To isolate Pseudomonas aeruginosa from various infections like Burns, CSOM, ventilator associated pneumonia and Catheter associated Urinary tract infections.
2. To find the strain similarities of Pseudomonas by antibiogram, Diene's Mutual inhibition test and Random amplified polymorphic DNA study.
3. To compare the three methods and to evaluate the easy, cheap, and the most suitable method which can be adopted in small laboratories at times of small outbreaks.

REVIEW OF LITERATURE

The organism was originally described by **Gessard in 1882** as Bacillus pyocyaneus and **Sedilot in 1850** identified blue green discharge from surgical dressings. Following this, **Luke (1882)** first isolated rod shaped organism present in blue green pus. The genus pseudomonas was proposed by **Migula (1894). Den Dooren de Jong (1926)** discovered the extraordinary mineralizing capacity of pseudomonas.

Stainer[62] et al 1966 classified pseudomonas species based on phenotypic characteristics with emphasis on the utilization of various compounds as sole of carbon and energy sources. Later this was followed by means of an estimation of the ribosomal RNA (rRNA) and similarities using rRNA – DNA hybridization as an analytical criterion by (**Palleroni[49,50] et al. 1973**). Palleroni explained 160 species within Pseudomonas genus. Only 12 species are of clinically importance. **Sikorski, J 2005** described that Pseudomonas stutzeri has atleast 18 Genomovars (or) Biovars. 1 and 2 genomovars are found in clinical isolates and others are found in plants, soil and marine sediment. Pseudomonas fluorescens was divided into biotypes A to G; biotype B

was pseudomonas marginalis, biotype D and E were Pseudomonas chlororaphil, Pseuodomonas aureofaciensrespectively. **Elomari[15], M. et al** explained that Pseudomonas putida consists of biovars A and B, biovar A regarded as typical as P. Putida, biovar B closely related to P. fluorescens.

Sugawara and Nikaido[65] (1992) identified Omp F channel properties. P. aeruginosa whose outer membrane could be considered basically as modified enteric outer membrane except that it lacks these classical porins of Omp F and OmpC.

Manaia and Moore,[38] 2002 described recently a thermo tolerant species with the name of P. thermotolerans and they can utilize an extraordinarily varied range of nutrients.

Plotkin[51] and Austrian 1958 explained, SS P. putida is commonly found in soil, water, plants, and antiseptics medications. **Bruch[7] 1971& Jersen, 1965**, identified it in ventilators**. Farmer[18] 1970** denoted Pseudomonas are found in processed foods. **Bergan[5] 1975, Cho[10] 1975** identified it from hands of health professional. **Hoadlly and**

Mccony[23], 1968 said that Pseudomonas can also colonise animals and human. They can spread among individuals.

In hospitals, among the species, the P.aeruginosa is a formidable opportunistic pathogen, and it is found in infection of immunocompromised patients like those with burns, neonates, AIDS and Cancer. **Green S.K.[20] et al** described Pseudomonas aeruginosa is a hardy saprophyte which can be isolated from a wide variety of environmental sources. **Gregory D.W. et al** explained its ability to persist in moist environments and moist equipments (eg.humidifiers) in hospital wards, bathrooms, and kitchens and give particular importance in cross infection Control.

Craigg.J[11], Yoshikazu Sakagami[68], 1989 described that the organism is resistant to and multiply in, many of the disinfectants and antiseptics like Benzalkoinium. **Russel A.D et al** observed that the organism is present in Gluteraldehyde commonly used in hospitals. **Stern G.A[63], et.al** described that it can become contaminant in Pharmaceutical preparation and may cause ophthalmitis following the faulty chemical sterilization of contact lenses and **S.Block[59], et al**

explained that the organism present in contaminated medicament. **Beers S.L** explained Pseudomonas aeruginosa could be recovered from body sites that remain moist such as outer canal of ear of children who swim frequently. **Ronald[57]. A,** et al described about endogenous infection particularly of Urinary tract by catheters.

Koh etal[30], Santha K.Murthy et al identified that Healthy carriers usually harbour strains in the Gastro intestinal tract, but in the open community Carriage rate seldom exceeds to 10%. In contrast, acquisition of P.aeruginosa in a hospital is rapid and upto 30% of patients may excrete the organism within 2 days of admission.

Pruitt[53], B.A.et al described that patients with burns are especially at risk. The presence of the organism in ward air, dust and in eschar shed from the burns suggests that infection can be air borne.

Deirdre[14] church et al informed transmission might occur directly via the hands of medical staff or indirectly via contaminated apparatus. **Jean Chastre, etal** described severely Burnt Patients and those with chest injuries who require ventilator for artificial ventilation

are very much susceptible particularly after (or) during treatment with broad spectrum antibiotics.

Hickey MM, etal described pulmonary infection frequently precedes septicemia, which is associated with high mortality. Chronic Pseudomonas lung infections are a major cause of morbidity in Cystic fibrosis patient.

Chastre J.[9] et al explained modern Intensive care unit could be difficult to clean, and there be little doubt that infection can be spread by the source. **Price d, et al & Salmon p et al** explained the warm moist air condition under which p. aeruginosa thrives are ideally met in poorly maintained whirl pools .Ear infection and an irritating follicultis may be acquired from swimming pools& hot tubs **(Ratnam et al)[55].**

Epidemics of genital tract infection can occur in new born, an, young infants in maternity units and pediatric wards, and may result from contaminated milk.

Palleroni[50] 1970, shinoda and okamato[60] 1977 first described the capacity of Pseudomonas aeruginosa of the swarming surface of solid media as in the case of P.stutzeri,which may be due to lateral flagella. They are involved in propelling the cell over the surface of solid media.

Thilokohler[67] described swarming of P.aeruginosa is dependent on cell-to-cell signalling and requires flagella and pili. Glutamate, Aspartate, Histidine, Proline that provides sole source of nitrogen induced swarming.

Kohler[31], 2000 explained that Rhamnolipid act a biosurfactant. Las & Rh1 encode auto inducer synthases responsible for synthesis of Biofilm (3 Oxa – C 12 & CH homoserine lactone and C4 homoserine lactone. **Manvi Sharma[39], 2002** explained that Alginate (Mannuronic & Glucuronic acid) is a mucoid polysaccharide; slime substance forms the matrix of pseudomonas biofilm, which anchors the cells to environment and in medical instruments. It protects the bacteria from host defenses such as PMN, Lymphocytes. Biofilm of mucoid strains of cystic fibrosis , diffuse bronchiolitis, endotracheal tube intubation tube covering are less susceptible to antibiotics.

P. aeruginosa, P. Putida, P. fluorescens are pigment producing bacteria.

King[29], Ward, Raney et al, 1954 first found **Pseudomonas Agar p. P.** aeruginosa produces pyocanin, greenish blue in colour due to phenazine pigment. **Pseudomonas Agar f.** P.fluorescens, P.putida **produced** fluorescin (or) pyoverdin, yellowish green discharge. **Meader[40] 1925** identified pyorubin a red water-soluble pigment characteristics of B.pyocyaneus. **Viloyas Allored, 1986 et al** experienced Lipid A moiety of Pseudomonas LPs (endotoxin) has exactly the same mechanism of action as the diphtheria toxin. It is regulated by exogenous iron. It has necrotizing activity at the bacterial colonization. It produces more virulent form of pneumonia and septicemia.

M. J. Bjorn, 1979 et al explained **Exoenzyme S** is an exotoxin. It is produced by P. aeruginosa in burned tissue and bacteremia. It impairs the function of phagocytes.

RM Berka[5] and ML Vasil, 1982, et al described **Phospholipase C was** one of the hemolysins breaks down lipids. It contribute to invasion through their cytotoxic effects of eukaryotic cells. It produces

breakdown of surfactant lead to lung collapse. **Kazuyuki Morihara[27] 1977**, et.al explained Alkaline Protease interferes with fibrin formation and will lyse fibrin, together with elastases destroy the ground substance of the cornea. **Leid[32] JG, 2005, et al** described that Mucoid strains produce an exopolysaccharide (Alginate), which has an attachment to tracheo bronchial mucin (N- acetyl glucosamine).

Anderson and Wood, 1969 clarified that all Pseudomonas have a functional Tricarboxylic acid cycle and can oxidize substrates completely to CO_2.**Enter and doudoroff 1952, Kerstersand Delay, 1968 et al** found Entner doudoroff pathway, degrades most hexoses. **Banerjee, 1989**), **Phibbs, 1988 et al** described Glyceraldihyde 3 – phosphate is recycles through pyruvate via lower EMP pathway. **Hederberg M[22] 1969 & Camphell 1988 et al** identified that Cetrimide has been used for the isolation and presumptive identification of P. aeruginosa from clinical specimens. **Fonseca[19] 1986** explained inhibition of some strains of P. aeruginosa from cystic fibrosis patient's sputum specimens have been reported to occur with the use of selective agar containing cetrimide 200 mg/ lt and nalidixic acid 15 mg/lt.

Typing of an individual strain is a major factor in epidemiological investigations.

Typing methods are

1. Biotyping
2. Antiobiotic Sensitivity Typing
3. Serotyping
4. Bacteriophage typing
5. Bacteriocin Typing
6. Modified Diene's mutual Inhibition Test
7. Random Amplified Polymorphic DNA Study

Charles D. Brokopp (1977) et al used 19 Pseudomonas serotypes for typing. 19 Group specific heat stable 'O' antigens and 2 heat labile H Antigens are recognized on the basis of standard slide agglutination test by Pitt (1988). More than 90% clinical strains can be typed by 'O' sero typing. In cystic fibrosis mucoid variants 'O'Ag are marked by 5%.

Hancock[21], 1982 et al described that cross-reaction of the serotype O' strains. They are expensive as Monoclonal antibodies against P.**aeruginosa** outer membrane antigens are used. Serotyping discrimination is improved by identification of H antigens.

Unfortunately the procedures for H antigen typing, using sera prepared against purified flagella are out with the scope of many laboratories. **Fyfe 1984 et al** identified three types of pyocins produced by P. aeruginosa. These are R, F and S.

R, F Pyocins are particulate and S pyocin is non-particulate. 13 indicator strains (1-8 and A to E) are used to identify the inhibition of the test strain (**Govan 1973 et al).** 13 indicator strains, 105 main types, 25 subtypes are recognized using **Govan (1973) strain**. Two methods are used 1. Cross-streaking method 2. Modified spotting method. S pyocin shows zone of Inhibition extending beyond the Area of original growth. R and F pyocin shows restricted zone of Inhibition (**Srinivas et. al 2000**). Pyocin typing requires a period of 24 hr to achieve a result but provides adequate discrimination of which to base more confidant epidemiological judgment.

Bosko Postic and Maxwell Finland[6] (1961) et al described phages to the typing of strains of P. aeruginosa from clinical infections using 7 phages. 1ml of 5-hour trypticase soy broth culture of was flooded on a trypticase soy agar plate and incubated at 37^{o}C after 25

minutes. 1ml of concentrated phage suspension containing 10^8 phage particles were poured over the surface. Incubated 16-18 hours at 31^oC confluent lysis was as read as +++, 20 – 30 clear plaques as ++ less than 20 as +, no lysis - negative. Similarity of phage pattern of different isolates from same patient suggestive the phage typing of P. aeruginosa prove useful for epidemiological purpose.

Bale and Hollis described that Swarming Proteus strains exhibit the Diene's phenomenon, the mutual inhibition of swarming and this forms the basis for precise method of differentiation among such strains. **Diene's L. 1946 et al, F.W. Hickman,[13] and J.de. Louvois et al** identified test organisms are isolated on to the surface of an agar plate and those that show no line of demarcation in area where the swarming growth meet are regarded as identical, if line of demarcation is identified it is different. Here genetically similar strains are seen in blood agar are clear line of demarcation due to point of inter section of swarming growth.

K.A. Bettel Heim (1984), described colony in compatibility studies of enterotoxigenic Escherichia coli O126 isolated during an out

break with the help of Diene's mutual Inhibition. **Munson et al[45] (2002)** proposed a modified Diene's test for the epidemiological characterization of p. aeruginosa. It is a simple and useful method for epidemiological typing of clinical samples of P. aeurginosa. Colonies of P. aeruginosa usually have a spreading morphology on blood agar media and have capability of adhering to central venous catheters. **Mahenthralingam E[37]. 1996 et al** described RAPD – PCR was a means of creating a biochemical fingerprint of organism. In standard PCR, known sequence of an organism's genome was amplified, but in RAPD – PCR random primer sequences may be used where a specific genome sequence is not known. Random Parts of the organism's genome are produced which are expected to be identical among related species and thus similar banding pattern should be produced in gel electrophoresis. This technology is quite useful in typing strains of bacteria in nosocomial infection.

Ogle. J.W. et al[48] described an Exotoxin A gene from Pseudomonas that was isolated through RFLP. **J.M. Janda 1987 et al** identified Pilin gene by RFLP and demonstrated in all cystic fibrosis patients. RFLP was proved superior to all phenotypic methods for

typing P.aeruginosa. Tenover et al explained PFGE is used for typing virtually any bacterial species and its high power. There can be more than three band differences among Isolates typed by PFGE and considered epidemiologically to be from same strain, and if there are three (or) few banding differences between two isolates, they should be considered to be from the same strain, as such differences are likely be due to one genetic divergent.**Curran B[12], 2004, et al explained MULTILOCUS SEQUENCE TYPING** is the most highly discriminatory among genetic typing tools. The method entails PCR amplification of specific genes and the sequencing of the gene products.

Regarding antimicrobials Beta – lactams (Piperacillin, Piperacillin TazobactumCeftazidime, Cefepime, Ticarcillin, Ticarcillin – clavulonate), Carba penems(Imipenam,Meropenem), aminoglycosides (Gentamycin, Tobramycin, Amikacin and Fluoroquinones (Cipro floxacin) are used as drugs of choice for Pseudomonas.

Marilee D. Obstrich[47], 2005 et al described emergence of multi drug resistant Pseudomonas isolates during therapy in 27 – 72 % of patients. **Cao B, Wang H[8]., 2004 et al** described Prolonged, hospital

stay, more than 7 days prolonged ant microbial therapy and mechanical ventilator for more than 48 hours are risk factors for colonization. **Liver more DM[33,34,35], 1995 et al** described Depression AmpC – Beta lactamases are mainly responsible for reduction in susceptibility to Betalactum antibiotics.

Studemeister[64] AE, 1988, et al described the loss of Opr D an outer membrance porin that forms narrow trans membrane channel accessible to carbapenems. **Pole K. 2005 et al** described mutations to topisomerase II and IV enzymes confer resistance to amino glycosides. **Evans ME[16], 1999, et al** described Polymyxin resistance occurs due to Pmr A gene in presence of low magnesium concentration which modifies LPS resulting in reduced binding affinity of colistin. An upregulated efflux system Mex AB – Opr M has been reported to decrease susceptibility to Penicillin ,Cephalosporin, Fluoro quinolone. Mex CD – Opr J, Mex EF – Opr N has been associated with resistance to Fluoro quinlons and some Beta lactams. **Pumbwel[54]. Piddockl J v, 2006 et al** described Mex xy – Opr M system associated with resistance to amino glycosides.Combination therapy may be efficacious including antipseudomonal beta lactam with an aminoglycoside (or) Fluroquinolone (or) Colistin with Rifampicin.

MATERIALS AND METHODS

This study on the **'EVALUATION OF VARIOUS METHODS IN THE IDENTIFICATION OF STRAIN HOMOLOGY AMONG PSEUDOMONAS ISOLATES'** involved the collection of 183 samples from Burns ward, IMCU, ENT OP and General Medicine ward at Govt. Rajaji Hospital during a period of 3 months from June2007 to September 2007.

The inclusion criteria of infections in this study were

1. Abscess of skin
2. Infections of burn wounds.
3. Chronic suppurative Otitis media
4. Ventilator associated pneumonias
5. Catheter associated Urinary tract infections of more than 1week.

The exclusion criteria were

1. Post operative and other wound infections
2. Acute Suppurative Otitis media
3. Pneumonias not associated with ventilator
4. Urinary tract infections not associated with catheters and catheter related UTI's less than 1 week.
5. Patient with DM, Malignancy, HIV & other cause of Infectious disease.

The various specimens collected in the study were aspirated Pus and **Swabs** from wound and ear infections, **Sputum** from ventilator associated pneumonias, and **urine** from catheter related urinary tract infections.

The study was commenced after obtaining approval from the Institutional Ethical Committee at Govt. Rajaji Hospital, Madurai.

Specimen Collection and Transport

The specimens from the wounds were collected either as **Pus** or a **Wound swabs**. For collecting pus from the abscess, the area was cleaned well with sterile saline and with a sterile syringe; Pus was aspirated by puncturing the centre of the abscess and drained into a sterile test tube. For taking wound swabs, pre sterilized wound swabs were used. The area was wiped with sterile saline and the swab moistened with the Stuart Transport Media and was introduced into the central necrotic area of the wound without touching the edges of the wound, rotated well, and removed carefully without touching the sides. The swab was immediately introduced into a sterile test tube with transport media and the mouth closed tightly.

For taking **Ear swabs** in Chronic Suppurative Otitis media, the crust surrounding the outer canal was wiped well with sterile saline, and

the sterile swab was introduced carefully into the canal, rotated firmly and removed without touching the surrounding areas. The swab was immediately introduced into a sterile test tube with transport medium.

For collecting **Sputum**, Sterile universal containers were used. Patient was given suitable instruction to collect specimen to rinse and the patient was asked to cough deeply and to bring forth the purulent sputum from within the lungs. The lid of the container was tightly closed without any leakage.

From catheterized patients, about 20 ml of **Urine** was collected in a sterile universal container directly from the catheter. The catheter just above the site of drainage was cleaned before collection. No preservative was added to the sample.

All the samples collected in the hospital were transported to the laboratory within 1-2 hrs. of collection. The swabs were transported in transport media. The request forms giving the details of age, sex, I.P.no, ward, diagnosis, date of collection etc. accompanied each specimen.

As soon as the specimens reached the laboratory, the request forms and the corresponding specimens were verified. The specimens were also examined macroscopically. The pus was examined for any peculiar odour, colour and consistency. The swabs in the transport

media were examined for the purulence, odour and the colour changes. The sputum was examined for purulence, odour , blood tinging and viscosity. The urine was examined for the turbidity or for the presence of deposits or suspended materials.

The suitable samples were identified and subjected to Gram staining. The samples showing gram negative bacteria were streaked on **Nutrient Agar Plate, Macconkey Agar**, **5% Sheep Blood agar** and incubated at 37 deg C over night. On the next day, the colonial morphology, pigmentation and the odour were noted. The organisms showing irridiscent colonies with mawkish odour on nutrient agar, Beta hemolysis with serrated edges on Blood agar were subjected as **Psuedomonas species**. They were further confirmed by bio chemical tests like Oxidase, Indole, Triple sugar iron agar, Citrate, Urease, Catalase, Oxidation- fermentation test and Acetamide test. The motility of the organism was confirmed by Hanging Drop method. The organisms which were showing indole negative, non fermentation with glucose, lactose and sucrose, Positive reactions for Citrate, Oxidase, Urease, Acetamide and Catalase, Oxidative utilization of glucose in

O-F medium and Motility by hanging drop were identified as **Pseudomonas aeruginosa**.

The sensitivity and resistant pattern of antibiotics for this organism was studied by the Kirby Bauer technique. Lawn culture was prepared on the surface of Mueller Hinton Agar plate and antibiotic discs of 6 mm diameter with the required concentrations were placed on the surface of the plate 20-25 mm away from the plate rim. A total of 8 discs were placed on a 100mm diameter plate. The plates were incubated over night and the grades of sensitivity were recognized as sensitive and resistant by comparing the diameters of the inhibition zone with the critical zone diameter in Kirby bauer chart. The resistant isolates were noted.

The identified Pseudomonas isolates were further screened by the following methods to identify the similarity & relatedness of strains :

1. Modified Diene's Mutual Inhibition Test

In this test, capillary tubes were aseptically sectioned in 1 cm. segments in biological safety cabinet. Pseudomonas aeruginosa strains were grown separately 5% sheep blood agar plates and 3 capillary tubes

were placed in each plate and incubated at 37degC for 24-48 hrs. When full growth of organism was noted on the tubes, the tubes were removed from the plates. Fresh 5% blood agar plates were prepared and 3 capillary tubes from 3 different isolates were placed over each fresh plate in such a way that the three tubes form an equilateral triangle on the surface of each fresh plate. All the plates were incubated in ambient air for 48 hrs. After 48 hrs., the plates were examined for intersection of colonial growth from the side tubes with the base tube which had standard strain growth. If there was no clearly visible line of demarcation, it was interpreted as indicating **high degree of relatedness (positive).** If there was an observable line of demarcation, it was interpreted as **unrelated (negative test)**. The isolates which showed high degree of relatedness with each other were taken as similar strains. Similar isolates from one plate were compared with from the other plates by keeping one capillary tube of similar strain in a 5% sheep blood agar plate with two other capillary tubes of similar strains. If there is relatedness between these 3 strains, they were taken as similar strains. Thus isolates no. **4, 5, 6, 22, 23, 25, 27, 28, and 30** showed relatedness to each other.

2. Random Amplified Polymorphic DNA typing:

Cell pellet for DNA extraction was prepared by inoculating 4-5 colonies from each Psuedomonas isolate into 15 ml. of nutrient broth , incubating at 37*c overnight and centrifuging at 4000 rpm for 20 min. The cell pellet was resuspended in 100 microlitre of DNA extraction solution kit (Genei, Bangalore) and the extraction continued following the manufacturer's instructions. The DNA was dissolved in 200 microlitre of enzyme free double distilled water, RAPD was run using PCR master mix following the manufacturer's instructions. The thermal cycler was run for initial denaturation at 94^{o} for 4mt, followed by 45 cycles of denaturation at 94^{o} for 1 mt, annealing at 36^{o} for 1 mt, elongation at 72^{o} for 2 mts, followed by final elongation at 72^{o} for 5 mts. The arbitrary RAPD primed Primers were screened for optimal amplification and primer A7 (5'C AGC CAG 3') was used. The amplified DNA fragments were visualized in agarose gel for the band pattern. The molecular weight of the bands produced by the isolates in agarose gel were viewed for similarity. In this study, the isolates no. **4, 5, 6, 22, 25, 27, and 28,** showed similar bands with molecular weight as follows:

1st band	-	807 bp
2nd band	-	470 bp
3rd band	-	453 bp
4th band	-	393 bp
5th band	-	323 bp.

Similar strains identified by their resistance to antibiotics, by the Diene's Mutual Inhibition test and Random Amplified Polymorphic DNA typing were compared and results taken up.

RESULTS

A total of 183 Samples obtained from patient admitted in the burns ward, IMCU, General Medicine ward and OP of ENT were Processed for the isolation of P. aeruginosa and similar strains were identified by three different methodologies 1) **Antibiotic Susceptibility test. 2) Diene's mutual Inhibition test 3) Random Amplified Polymorphic DNA Study** and analysed for the most suitable cheap & simple method, for identifying similar strains of Pseudomonas in small laboratories.

Age wise distribution of samples showed that in Burns, 5 out of 74 (7%) were in age group 0-1yr, 10 out of 74 (14%) in 1-20yr, 45 out of 74 (61%) in 21-40yr, 14 out of 74 (19%) were in age group more than 40yrs.

Similarly in Ventilator associated pneumonia, no case was observed in the age group 0-1 yr., 3 out of 20 (15%) were in age group 1-20yr, 11 out of 20 (55) in 21-40yr,and 6 out of 20 (30%) were in age group more than 40 Yrs.

In CSOM, 4 out of 57 were in the age group 0-1yr (7%), 25 in 1-20 yrs (44 %), 22 in 21 – 40yrs (39%), and 6 (11%) in age group more than 40 yrs.

In Urine samples no case was in age group 0-1yr (0%), 2 out of 32 in 1-20 yr (7%), 17 in age group 21-40 yr (53%), 13 in age group more than 40yrs (41%). This is given in table no.1.

Table- 1

Age wise distribution of samples

SL. No.	Infection	Total No	Age group in years							
			0-1 Total	%	1-20 Total	%	21-40	%	> 40 Total	%
1.	Burns wound Infection	74	5	7%	10	14%	45	61%	14	18%
2.	CSOM	57	4	7%	25	44%	22	38%	6	11%
3.	Ventilator associated Pneumonia	20	-	-	3	15%	11	55%	6	30%
4.	Catheter associated UTI	32	-	-	2	6%	17	53%	13	41%
	Total	183	9	5%	40	22%	95	52%	39	21%

Thus it was found that 52%(95 out of 183 samples) samples collected were in the age group 21-40 yrs, and maximum number of samples were collected in the burns ward.

All the 183 samples were analysed wardwise and sex wise, and it was found that 74 out of 183 were from Burns ward (40%), 26 out of 183 were from IMCU (14%), 57 out of 183 were from ENT OP (32%), 26 out of 183 were from Medicine ward (14%). (Table.2)

The sex wise distribution showed that 36 out of 74 (19%)from Burns ward were male and 38 out of 74(22%) from Burns ward were females. Similarly 12 out of 26 from IMCU were males (6%), 14 out of 26 from IMCU were female (8%). Out of 57 from ENT, 22 were males (12%) and 35 were females (19%). Out of 26 from medicine l0 were males (5%), 16 were females (9%). This is given in Table2.

Table - 2
Distribution of samples ward wise and sex wise

Sl.NO	Name of ward	Total		Males		Females	
		NO	%	TOTAL	%	TOTAL	%
1	Burns	74	40%	36	19%	38	*22*%
2	IMCU	26	14%	12	6%	14	8%
3	ENT OP	57	32%	22	12%	35	*19*%
4	Medicine	26	14%	10	5%	16	*9*%
	Total	183	100%	80	42%	103	*58*%

It was found that 58% samples collected were from females especially from burns, ENT and Medicine.

The samples collected were analysed as per Gram reaction after isolation and it was found that 30 out of 183 (16%) were Gram positive organism, 153 (84%) were Gram negative organism, and analysis of Gram negative organism showed 72 (47%) were Pseudomonas aeruginosa. 37 (24%) were E.coli: 18 (12%) were Klebsiella species and16 (10%) were Proteus species. This is given in Table.3

Table-3

Bacteria Isolated from 183 samples

Sl. No.	Total	Organisms				Gram Negative Organism							
		Gram Positive Organism		Gram Negative Organism		Pseudomonas		E.coli		Klebsiella Species		Proteus Species	
		Total	%	Total	%	Total	%	Total	%	Total	%	Total	%
1	183	30	16%	153	84%	72	47%	37	24%	18	12%	16	10%

It was found that 84%(153 out of 183) samples collected were Gram negative organisms and 47%(72 out of 183) among them were Pseudomonas spp.

Age and Sex wise distribution of Pseudomonas showed that 1 out of 72 (0.5 %) was isolated in 0-1year age group and the isolate was from a female, 14 out of 72 (20%) in 1-20 age group, and 6 (8%) isolates were from males and 8 (11%) isolates were from females. 35 out of 72 (49%) were isolated from 21-40 year and 14 (19%) were isolated from males, 21 (29%) were isolated from females. 22 out of 72 (31%) were isolated from more than 40 years, and 11(15%) were isolated from males, 11 (15) were isolated from females. This is shown in table 4

Table 4

Age wise & Sex wise Distribution of Pseudomonas

Total: 72 no.

Sl. No.	Age in years	No	%	Male		Female	
				No	%	No	%
1	0-1	1	0.5%	-	-	1	0.5%
2	1-20	14	20%	6	8%	8	11%
3	21-40	35	49%	14	19%	21	29%
4	> 40	22	31%	11	15%	11	15%
Total		72	100%	31	43%	41	57%

It was observed that Pseudomonas isolated for 57%.,out of which 29% were from burns.

The ward wise distribution of Pseudomonas showed that 37 out of 72 Isolates were from Burns ward (51%), 8 out of 72 isolates from IMCU (11 %), 20 from ENT OP (28%), and 7 Medicine ward, (10%) and this is shown in Table 5

Table –5

Ward wise distribution of Pseudomonas aeruginosa

Total: 72 no.

SL. No.	Name of Ward	No: of Pseudomonas	% of pseudomonas
1.	Burns	37	51%
2.	IMCU	8	11%
3.	ENTOP	20	28%
4.	Medicine	7	10%
	Total	72	100%

It was observed that 51 % of pseudomonas isolates were from burns ward.

Specimen wise distribution of pseudomonas showed 57 out of 72 Isolates were from Pus and swab (79%), 5 out of 72 were from sputum (7 %), 10 out of 72 were from urine (14 %), and this is shown in table 6.

Table 6

Specimen wise Distribution of Pseudomonas

Total: 72 no.

Sl.No.	**Name of specimen**	**No**	**%**
1.	Pus, swab	57	79%
2.	Sputum	5	7%
3.	Urine	10	14%
	TOTAL	72	100%

It was found that 79% Pseudomonas isolated were from Pus samples.

Antibiotic Susceptibility pattern of Pseudomonas showed 17 out of 72 isolates (23.5%) were resistant to more than 6 antibiotics(MDR). Among them, 9 (12.5%)were resistant to Carbencillin, Tobramycin, Ciprofloxacin, and Piperacillin – tazobactum which were the antibiotics used in the hospital during the study period. Among them 6 were isolated from Burns ward (67%), 2 were from ENT OP (22%), and 1 was from IMCU (11%). This is shown in table –7

Table-7

Antibiotic Susceptibility Pattern of Pseudomonas aeruginosa

Total: 72 no.

SL. No.	**Name of Ward**	**Total**	**%**	**> 6 Antibiotics Resistant**		**4 Antibiotics Resistant**	
				Total	**%**	**Total**	**%**
1	Burns	37	51 %	9	12.5%	6	8.3%
2	ENT	20	27%	5	7%	2	3%
3	IMCU	8	12%	2	3%	1	0 .5%
4	Medicine	7	10%	1	1%	_	_
	Total	72	100%	17	23.5%	9	12.5%

It was found that 23.5% showed Resistance to more than 6 antibiotics, among which 12.5% were resistant to Carbencillin, Tobramycin, profloxacin, and Piperacillin – Tazobactum and it was 8.3% in burns ward.

Diene's Mutual Inhibition test was positive in that 9 out of 72 samples (12.5%), showing similarity with each other. Among them, 6 were isolated from burns ward (8.3%)and 3 from ENT OP (4.2%), This is given in table no-8,9

Table 8

Diene's Mutual Inhibition test

Total: 72 no.

Sl. No	Name of ward	Positive		Negative	
		Total	**%**	**Total**	**%**
1.	Burns	6	8.3%	31	43.2%
2.	ENTOP	3	4.2%	17	23.6%
3	IMCU	-	-	11	15.2%
4	G. Medicine	_	_	4	5.5%
Total		9	12.5%	63	87.5%

It was found that 12.5% were similar strains and 8.3% of them were from burns ward.

Table 9

Isolates vs Diene's positives

Total: 72 no.

Sl.No.	Ward	Similar Strain	Relative strain confirmation
1	Burns	Isolate 4, 5,6	Isolates 5,25,27 (relatedness)
		Isolates 25,27	Isolates 5,23,30(relatedness)
		Isolates 23,30,	_
2	ENT OP	Isolates 22,28	Isolates 22,28,6(relatedness)
3	IMCU	-	_
4	Medicine	_	_

It was found that the isolates no.4, 5,6, 22,23, 25,27,28, and 30, were similar strains.

In Random amplified polymorphic DNA study, it was found that 7 out of 72 samples were positive (9%). Among them, 5 were isolated from Burns ward (6.9%) and 2 were isolated from ENT OP (2.7%), **This is given in Table no. 10**

Table-10
Random Amplified Polymorphic DNA STUDY

Total: 72 no.

Sl. No	Name of ward	Similar Band		Dissimilar Band	
		Total	%	Total	%
1.	Burns	5 Isolates 4,5,6,25,27	6.9%	32	44.4%
2.	ENTOP	2 Isolate 22,28	2.7%	18	25%
3.	IMCU	-		11	15.3%
4.	Medicine	_		4	5.5%
	Total	7	9.7%	65	90.3%

It was found that 9.7% showed positive RAPD and 6.9% of them were from burns ward.

The phenotypic characters of the pseudomonas identified by antibiotic Susceptibility test and Diene's Mutual Inhibition test were compared with the genotypic characters identified by RAPD and it was found that 7 isolates (9%)showed similar banding pattern in RAPD, 9 isolates (12.5%)showed relatedness with each other by Diene's and all the nine isolates (12.5%) were resistant to Carbencillin, Tobramycin, Piperacillin- Tazobactam, Ciprofloxacin. This is given in table no.11

Table-11

Comparison of Three Methodologies

Sl.No.	**Antibiogram**		**Modified Diene's Mutual Inhibition Test**		**RAPD**	
	Total	**%**	**Total**	**%**	**Total**	**%**
1.	**9**	**12.5%**	**9**	**12.5%**	**7**	**9%**

Thus it was found that Modified Diene's Mutual Inhibition test and the antibiotic susceptibility test which are the phenotypic tests had detected equal number of related isolates whereas RAPD which is a genotypic test detects less number of similar isolates . Among the two phenotypic tests, Dienne's Mutual inhibition test is easy to perform, and interpreted easily and it is also less expensive whereas Antibiotic susceptibility test is costlier and interpretation is difficult. Genotypic tests are expensive, need sophisticated equipments, and time consuming. Hence Dienne's Mutual Inhibition test is considered to be the most suitable method for identifying similar strains of Psuedomonas in small laboratories especially during epidemics.

DISCUSSION

The study on **'EVALUATION OF VARIOUS METHODS IN THE IDENTIFICATION OF STRAIN HOMOLOGY AMONG PSEUDOMONAS ISOLATES'** involved the collection of 183 samples from patients admitted in Burns ward, IMCU, and General Medicine ward and OP of ENT from Govt. Rajaji hospital, Madurai. The samples were processed for the isolation of Pseudomonas aeruginosa following standard techniques and the isolates were analysed agewise, sexwise and wardwise . They were further subjected to **Antibiotic susceptibility test, Diene's Mutual inhibition Test and Random Amplified Polymorphic DNA** for finding similar strains among them.

In this study, it was found that **52%** of samples were collected in the **age group 21-40 years**, (mean age 30.5 yrs)especially in the burns ward. Similar study by **Ahmed[1] et al** showed that **49.3 %** of their study population was in the mean age group 33. 3 yrs.which is in accordance with this study. **Faridaak[17] et al** in their study showed that **59.2 %** of their study population were from burns ward. The cause for the increased incidence of infection in burns ward may be attributed to the

loss of natural cutaneous barrier to infection, coagulated proteins and other microbial infections in the burn wound and combined avascularity of the wound which might lead to microbial infection.

In this study **58% were females especially from burns ward**. Similar study by **Pruit[53] et al** showed that their study population had **49.3 % females.** The most common mode of suicidal burns in our population is immolation because fire is the easiest access to females and momentary feelings will make them to go for easily accessible things. As suicidal burns are very common in our hospital, collection of more samples from burns ward in females is justified.

In this study**, 84% bacteria isolated were Gram negative**. **Akay ley[2]** et al in their study also demonstrated that **77.9%** organisms in their study were Gram negative. The normal flora colonizing the skin influences the infection. Although the Gram positive organisms colonise skin more abundantly, their influence will be there only during the first phase. The Gram negative organisms are the predominant inhabitants of the deeper wound because they have greater invasive potential than Gram positives.

In this study, among the Gram negatives,47% were Psuedomonas isolates. **Akayley[2] et al** also showed **50.4%** pseudomonas isolates. Psuedomonas is a very common Gram negative organism in hospital surrounding because of the obvious reasons that they are resistant to common antibiotics used in the hospitals, they survive well in moist environment and they even grow in antiseptic lotions. Hence isolation of more number of Pseudomonas is justified.

In this study, **57% females in the age group 21-40 years showed Pseudomonas in burns ward**. Similar study by **Farmer J.J**.[18] revealed that **52 % females** showed Pseudomonas. **Akay ley[2] et al** showed that **20-30 yrs**. was the common age group involved in the isolation of pseudomonas. **Anupurba Shamba[3]** also explained that the common age group involved was **16-40 yrs.** Since suicidal attempts are common in the age group 16-40 yrs. due to multivarious obvious reasons, and burns is the commonest mode of suicidal attempt, isolation of Pseudomonas in this age group is justified .

In this study**, it was found that 51% pseudomonas was present in burns wards**. **Mehta manjula[41] et al showed 51%** pseudomonas in burns infection which correlates with this study. Pseudomonas aeruginosa from the patient's endogenous intestinal flora and/or an environmental source is the most common cause of burn wound infection by this organism. It produces cell associated and extracellular virulent factors that mediate a number of processes including adhesin, nutrient acquisition, immune system invasion, leucocyte killing, tissue destruction and blood stream invasion. They also carry many intrinsic and acquired antimicrobial resistant traits that makes infected wound difficult to treat. Also, the biofilms which are the surface attached aggregates of microbes act as efficient barriers against antimicrobial agents and the host immune system resulting in persistent colonization and/ or infection.

In this study, **79% pseudomonas were isolated from pus**. **Anupurpa shamba[3]** et al in their study showed **69 %** pseudomonas were isolated from pus. The moist environment of pus suitable for the growth of Pseudomonas and the lack of neutrophilic response to tissue invasion made it possible for more isolation of pseudomonas in pus.

In this study it was proved that **23.5 % pseudomonas were resistant to more than 6 antibiotics and 8.3 % were resistant to carbenicillin, tobramycin, pipearcillin tazobactem, and ciprofloxacin**. **Obritsch**[47] et al demonstrated **24.3 % mutidrug resistance** and **Jung**[47] **et al** demonstrated **24%** multi drug resistance. Multiple drug resistance may be due to inappropriate therapy, delay in starting appropriate therapy, prolonged hospital stay or due to derepression of the chromosomal Amp-C beta- lactamases which reduce the susceptibility to beta lactum antibiotics.

In this study, by Diene's **mutual inhibition test, 8.3% isolates** showed relatedness with each other and all the isolates were from burns ward.. **Munson**[45] **et al** in his study **showed 9.2% related strains** in Pseudomonas by Diene's phenomenon but the isolates were from varied infections. But Munson used central venous catheters instead of capillary tubes, which were used in this study. As both the techniques give the same result, substituting central venous catheter by capillary tube is justified.

In this study, **6.9% isolates showed similar bands by Random Amplified Polymorphic DNA Study**. All the isolates were from burns ward. **Menon[42] et al** demonstrated **20% isolates** with similar bands by RAPD but the isolates were from ophthalmic cases only. **Maureen Campbell[37]** et al demonstrated 94 % isolates showing similar bands in cystic fibrosis cases. The variations in the isolation rates in the three studies might be due to the variation in the specimens.

SUMMARY

The study on **'EVALUATION OF VARIOUS METHODS IN THE IDENTIFICATION OF STRAIN HOMOLOGY AMONG PSEUDOMONAS ISOLATES'** showed that 52% of the samples collected were in the age group 21-40 yrs. mainly from burns ward and 58% were females. 84% samples collected were Gram-negative organisms and 47% were Pseudomonas aeruginosa. 57% females in the age group 21-40 yrs showed Pseudomonas aeruginosa. 51% Pseudomonas isolates were from burns ward and 79% Pseudomonas were isolated from pus.

The antibiogram showed that 12.5 % of the isolates were resistant to carbenicillin, tobramycin, ciprofloxacin and piperacillin-tazobactum which were the common antibiotics used during the study period and 9% were from burns ward. Diene's mutual inhibition showed that 12.5% isolates were related to each other and 8.3 % were from burns ward. RAPD showed that 9% showed similar bands and 6.9% of them were in the burns ward. The comparative study of the three methodologies

showed that antibiogram and Diene’s showed equal no. of related isolates whereas RAPD showed less number of isolates with similar bands. But Diene’s mutual inhibition test is a simple, less expensive procedure and is easy to interpret also. Hence it can be recommended for small laboratories for identifying similarity of strains of P.aeruginosa especially in times of small outbreaks.

CONCLUSION

The **'EVALUATION OF VARIOUS METHODS IN THE IDENTIFICATION OF STRAIN HOMOLOGY AMONG PSEUDOMONAS ISOLATES'** showed that

- **Pseudomonas aeruginosa was commonly isolated from Pus samples in burns ward in females of age group 21-40 yrs.**

- **The similarity of strains of Pseudomonas aeruginosa was identified by Antibiotic susceptibility test, Diene's mutual inhibition test and Random amplified polymorphic DNA test.**

- **The comparative study of the different methods, it was found that Diene's mutual inhibition test was simple to perform and cheap and may have utility in initial screening of Pseudomonas aeruginosa isolates in suspected common source outbreaks. This test is particularly appealing to smaller clinical microbiology laboratories with financial and technical constraints that may preclude use of molecular biology based methods in epidemiological investigations, and hence recommended.**

BIBLIOGRAPHY

1. **Ahmed m, Shahid hussain, Ibrahim Khan, Malik S.A.,** Pattern of Bacterial Invasion in Burn Patients at the Pakistan Institute of Medical Sciences, Annals of burns and Fire Disasters, March 2006, X1X, 1.
2. **Akayleh A.T**, Invasive Burn Wound Infection, Annals of Burns and Fire Disasters, Dec 1999,X11, 4.
3. **Anupurma Shama, Bhattacharjee amithaba, GargAtul, Senmaly Rajan,** Antimicrobial Susceptibility of Pseudomonas aeruginosa isolated from wound infections, 2006,51-4, 286-288.
4. **Beers, S.L., and T.J. Abroma**. Otitis externa review. Pediatr. Emerg. Care**2004.** 20,250 – 256.
5. **Bergan. T.** Epidemiological typing of Pseudomonas aeruginosa. In: Brown M.R.W.(ed). Resistance of Pseudomonas aeruginosa. London: John Wiley & Sons, **1975,** 182 – 235.
6. **Bosko Postic and Maxwell Finland**; Observations n Bacteriophage typing of Pseudomonas aeruginosa. NIH; **1961.**

7. **Bruch C.W.** Cosmetics: Sterility vs. microbial control. American performer and cosmetics, **1971**,86, 46 – 51.

8. **Cao B, Wang H, Sun H, Zhu Y, Chen M**. Risk factors and clinical outcomes of nosocomial multi–drug resistant Pseudomonas aeruginosa infections. **2004**, 57, 112-18.

9. **Chastre, J. and J.Y. Fagon.** Ventilator – associated Pneumonia. AM J. Respir, Crit. Care Med. **2002**,165, 867 – 907.

10. **Cho J.J. Schroth, M.N.** Ornamental plants as carriers of Pseudomonas aeruginosa. Phytopathology. **1975,** 65, 425 – 31.

11. **Cragg. J. and Andrews, A.V.** Bacterial contamination of disinfectants Br. med J. **1969**, 3. 57.

12. **Curran, B., D. Jonas, H. Grunfmann, T.Pitt, and C.G. Dowson.** Development of a multilocus sequence-typing scheme for the opportunistic pathogen Pseudomonas aeruginosa. J. Clin. Microbial. **2004,** 42, 5644 – 5649.

13. **Dienes, L.,** Reproductive process in Proteus cultures. Proc. Soc. Exp. Biol. Med. **1946**,63,265 – 270

14. **Deirdre Church, Sameer Elsayed, Owen Reid, Brent Winston, and Robert Lindsay' Burn Wound Infections,** Clin Microbiol Rev. 2006, April; 19(2): 403–434

15. **Elomari, M., L. Coroler, S. Verhille, D. Izard and H. Leclerc,** Pseudomonas monteilli sp. Nov., isolated from clinical specimens. Int. Syst Bacterial, **1997**, 47,846 – 852.

16. **Evans ME, Feola DJ, Rapp RP. PolymyXin B. Sulfate and Colistin:** old antibiotics for emerging multi drug resistant gram negative bacteria, Ann Pharmacother 1999, 33, 960-7.

17. **Faidaak H.A., Ballal S.G., Hegazi M., Al-Hoqail R., Al-Awad N.** - Saudi Arabia, Analysis and outcome of 835 burn injury admission to burn ward, King fahd hospital, Al-Kobhar, Saudi Arabia, Annals of Burns and fire disasters Dec1999,

18. **Farmer. J.J.** Pseudomonas in the hospital. Hosp. Pract. **1976,** 11. 63 – 70.

19. **Fonseca, K.A., K. Macdougll, and T.L. Pitt.** Inhibition of Pseudomonas aeruginosa from cystic fibrosis by selective media, J. Clin. Pathol. **1986,** 39, 220 – 222.

20. **Green, S.K. and Schroth**, M. N,. Agricultural plants and soil as a possible reservoir for Pseudomonas aeruginosa. Appl. Microbiol. 1974, 28. 987 – 91.

21. **Hancock, R.E.W., Wiezorek, A.A. et.al.** Monoclonal antibodies against Pseudomonas aeruginosa outer membrane antigens; isolation and characterization. Infect Immun, **1982,**37, 166 – 71.

22. **Hedberg, M.** Acetamide agar medium selective for pseudomonas aeruginosa. Appl. Microbial. **1969,** 17,481.

23. **Hoadley, A.W. and McCoy. E.** Some observations on the ecology of Pseudomonas aeruginosa and its occurrence in the intestinal tracts of animals. Cornell. Vet. **1968**,58. 354 – 63.

24. **Hollis, R.J., J.L. Bruce, S.J. Fritschel, and M.A. Pfaller.** Comparative evaluation of an automated ribotyping instrument versus pulsed field gel electrophoresis of epidemiological investigation of clinical isolates. Diagn Microbiol. Infect. Dis. **1999,** 34, 263 – 268.

25. **Holt, J. G. N.R. Kreig, P.H.A. Sneath, J.T. Staley, and S.T. Williams** Bergey's manual of determinative bacteriology, 9th

ed., **1994**, 93 – 94, 151 – 168. The William & Wilkins Co. Baltimore, Md.

26. **Jessen.O.** Pseudomonas aeruginosa and other green fluorescent pseudomonas. A taxonomic study. Copenhagen: Munksgard, **1965.**
27. **Kazuyuki Morihara And Hiroshige Tsuzuki,** Two extra cellular activities associated with virulence in pseudomonas aeruginosa Infection and Immunity Mar **1977**. P. 679 – 685.
28. **Kersters, K., M. Ludwig, P. Vancanneyt, P. De Vos, M. Gillis, and K.H. Schleifer.** Recent changes in the classification of the pseudomonas: an overview system. Appl. Microbiol. **1996,** 19, 465 – 477.
29. **King. E.O. Ward, M.K. and Raney. D.E.** Two simple media for the demonstration of pyocyanin and fluorescein, J. Lab Clin Med, **1954**,44, 301 – 7.
30. **Koh, A.Y., G. P. Priebe, and G.B. Pier.** Virulence of pseudomonas aeruginosa in a murine model of gastrointestinal colonization and dissemination in neutropenia. Infect. Immun. **2005**, 73. 2262 – 2272.

31. **Kohler, T., M. Michea – Hamzehpour, S.F. Epp, and J.C. Pechere,** Carbapenem activities against Pseudomonas aeruginosa; respective contributions of Opr D and efflux systems, Antimicrob, Agents Chemother, **1999,**43, 424 – 427.

32. **Leid J.G. Mucoid Strains of Pseudomonas aeruginosa J. Immunol. 2005** Dec 1; 175 (11) 7512 – 8.

33. **Livermore, D. M.**. Beta – Lactamases in Laboratory and clinical resistance. Clin. Microbiol, **1995,** Rev. 8: 557 – 584.

34. **Livermore, D.M.** Clinical significance of beta – lactamase induction and stable depression in Gram negative rods. Eur. J. Clin Microbiol, **1987,** 6, 439 – 445.

35. **Livermore, D.M.** Multiple mechanisms of antimicrobial resistance in Pseudomonad aeruginosa; our worst nightmare? Clin. Infect. Dis **2002,**34, 634 – 640.

36. **M. Camphell, Mahenthiralingam, E., and D.P. Speert,** Nonmotility and phagocytic resistance of pseudomonas aeruginosa isolates from chronically colonized patients with cystic fibrosis. Infect. Immune, **1994**, 62, 596 – 605.

37. **Mahenthiralingam, E., M. Camphell, J. Foster j.S. Lam, and D.P. Spreet,** Random amplified polymorphic DNA typing

of Pseudomonas aeruginosa isolates recovered from patients with cystic fibrosis. J. Clin. Microbiol **1996**,34,1129 – 1135.

38. **Manaia, C.M. and Moore, E.R.B.** Pseudomonas thermotolerans sp. thermotolerant species of the genus Pseudomonas. Int Syst Envol Microbiol, **2002**, 52, 2203 – 9.
39. **Manvi Sharma** – Swarming Bacterial – review article current Science vol 83. No. 6; 25 September.
40. **Meader. P.D., Robinson. G.H. and Leonard, V.** Pyorubin, a red water – soluble pigment characteristic of B. Pyocyaneus. Am J. Hyg, **1925,**5. 682 – 707.
41. **MehtaManjula, DuttaPriya, GuptaVarsha**, Bacterial isolates from burn wound infections and their antibiograms: An eight-year study, Indian Journal of plasticsurgry, 2007,40, 1, 25-28.
42. **Menon PK, Dr. Shiv Priya Eswaran, Dr. SS. Pant;** Random Amplification of Polymorphic DNA Based typing Pseudomonas aeruginosa. MJAFI **2003;** 59; 25 – 28.
43. **MJ Bjorn** Exotoxins activity of Pseudomonas aeruginosa, Infection and Immunity**, 1979** June 24(3), 837 – 842.

44. **ML Vasil, D. Kabat and BH Iglewski,** Structure – activity relationship of exotoxin of Pseudomonas aeruginosa: Infect Imun, **1977** April 16(1), 353 – 361.

45. **Munson. L. Micheal A. Pfaller, and Gray V. Doern** Modification of Dienes Mutual inhibition Test for Epidemiological characterization of Pseudomonas aeruginosa JCM, Nov. **2002,** 11, 40, 4285 – 4288,

46. **Niloyas Allured,** Exotoxin A activity of Pseudomonas PNAS , March **1986** ,83, 5 , 1320 - 1324.

47. **Obritsch MD, Fish DN, MacLaren R, Jung R**. National surveillance of antimicrobial resistance in Pseudomonas aeruginosa isolates obtained from intensive care unit patients from 1993 to 2002, Antimicrob Agents Chemother, **2004**; 48,4806 – 10.

48. **Ogle, J. W., J.M. Janda, D.E. Woods, and M.L. Vasil.** Characterization and use of a DNA probe as an epidemiological marker for pseudomonas aeruginosa, J. Infect. Dis **1987**,155, 119 – 126.

49. **Palleroni N.J., Kunisawa, R. et.al.** Nucleic acid homologies in the genus Pseudomonas, Int J. Syst Bacterial, **1973,**23. 333 – 9.

50. **Palleroni. N.J. Doudroff. M. et al.** Taxonomy of the aerobic Pseudomonas. The properties of the Pseudomonas stutzeri group. J. Gen. Microbiol. **1970**,60. 215 – 31.

51. **Plotkin. S.A. and Austrian. R.** Bacteriemia caused by Pseudomonas following the use of materials stores in solutions of a cationic surface-active agent AM J. Med Scien. **1958,** 235. 621 – 7.

52. **Poole, K.** Aminoglycoside resistance in Pseudomonas aeruginosa. Antimicrob. Agents Chemother, **2005,** 49, 479 – 487.

53. **Pruitt, B.A., Jr. A.T. McManus, S.H. Kim, and C,.E. Goodwin.** Burn wound infections: current status world J. Surg, **1988**, 22,135 – 145.

54. **Pumbwe L, Piddock LJV**. Two efflux systems expressed simultaneously in multidrug resistant Pseudomonas aeruginosa. Antimicro Agents Chemother **2000**; 44:2861 -4.

55. **Ratnam .S,** Whirlpool associated follicultis caused by Pseudomonas aeruginosa; report of outbreak and review JCM **1986** Mar; 23(3) 655 – 9.

56. **RM Berka and ML Vasil** cytotoxicty of Pseudomonas aeruginosa J. Bacterial. 1982, October; 12 (1); 239 – 245.

57. **Ronald, A.** The etiology of urinary tract infection traditional and emerging pathogens. Am J. Med ,**2002**,113 (Suppl. 1A): 145 – 193.

58. **Russel A.D.** Inhibition of EDTA – Lyzozyme lysis of Pseudomonas aeruginosa by glutaraldihyde Microbiol 13, 151 – 153.

59. **S. Block** Hand Hygine Disinfection, sterilization and preservative 5th edition 2001.

60. **Shinoda. S. and Okamoto, K.** Formation and function of vibrio parahemolyticus lateral flagell. J. Bacterial, **1977,** 129. 1266 – 71

61. **Sikorski, J., J, Lalucat, and W. Wackernagel.** Genomovers 11 to 18 of Pseudomonas stutzeri identified among isolates from soil and marine sediment. Int. Syst. E.vol. Microbiol **2005**, 55: 1767 – 1770.

62. **Stanier, R.Y., Palleroni, N.J. and Doudoroff, M.** The aerobic Pseudomonas: A taxonomic study. J. Gen Microbiol, **1966,** 43. 159 – 271.

63. **Stern, G.A.** Pseudomonas Keratitis and contact lens wear; the lens / eye is at fault. **1990,** Cornea 9 (Suppl. 1): S 36 – S 40.

64. **Studemeister AE, Quinn JP.** Selective imipenem resistance in Pseudomonas aeruginosa associated with diminished outer membrane permeability. Antimicrob Agents Chemother 1988; 32:1267-8

65. **Sugawara, E. and Nikaido, H**. Pore – forming activity of Omp A protein of Escherichia coli. J. Biol Chem, **1992,**267, 2507 – 11.

66. **Tenover, F.C., R.D. Arbeit, R.V. Goering, P.A. Mickelson, B. E. Murray F.H. Persing and B. Swamniathan**, Interpreting, chromosomal DNA restriction patterns produced by pulsed –field gel electrophoresis: criteria for bacterial strain typing J. Clin. Microbiol **1995,** 33: 2233 – 2239.

67. **Thilo Kobler –** Swarming of Pseudomonas aeruginosa JCB Nov 2000, 2. 5996

68. **Yoshkazu Sakagami;** Mechanism of Resistance to Benzalkonium chloride by Pseudomonas aeruginosa Applied and environmental microbiology Aug. 1989, P –8, 155, 2036 – 2040.

ANNEXURE

ACETAMIDE UTILIZATION

This test is used to determine the ability of an organism to use acetamide as the sole source of carbon. Bacteria that can grow on this medium deaminate acetamide to release Ammonia. The production of Ammonia results in Ph driven colour change of the medium from green to blue.

Method

Inculate acetamide slant lightly with a needle using growth from an 18– 24 hour culture.

Positive : Deamination of the acetamide resulting in a blue colour.

Negative : No colour change

QC : + P.aeruginosa

\- Stenotrophomas maltophila

PROFORMA

Name: **Age: Yrs**

IP/ OP NO: **Sex:**

DOA: **DOD:**

Ward: **Unit:** **Date:**

Full Residential address:

Socioeconomic status : Poor/ Moderate/ Well

History of Present Illness:

1. **Pus, Abscess From Burns Wound _____ Days**
2. **Ear Discharge _____ Days**
3. **Sputum _____ Days**
4. **Irritation of Urination_____ Days**

Clinical Diagnosis

Treatment Details:

Specimen Collected

1. **Pus**
2. **Urine**
3. **Sputum**

Date of collection :

Date of processing :

Date of Report :

Microbial Process:

1. **Direct Smear**
2. **Hanging drop**

Culture

1 **Nutrient Agar**

2. **Macconkey's agar**
3. **5% sheep Blood agar**

Biochemical Reaction

Oxidase **Catalase** **Indole** **Citrate** **Urease**

Acetamide **TSI** **OF- Glucose**

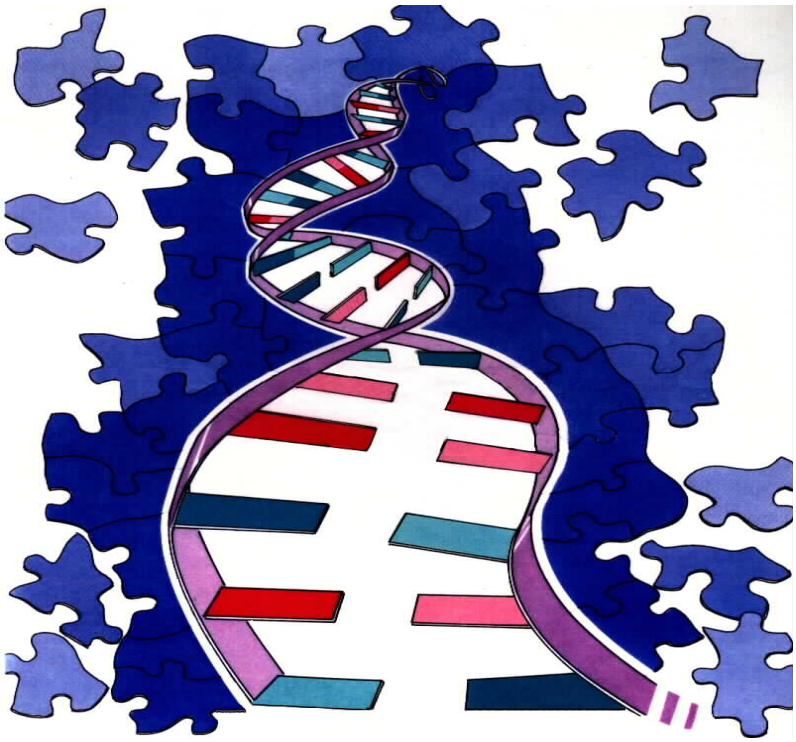
ANTIBIOGRAM

1. **Highly Sensitive**
2. **Moderately Sensitive**
3 **Resistant**

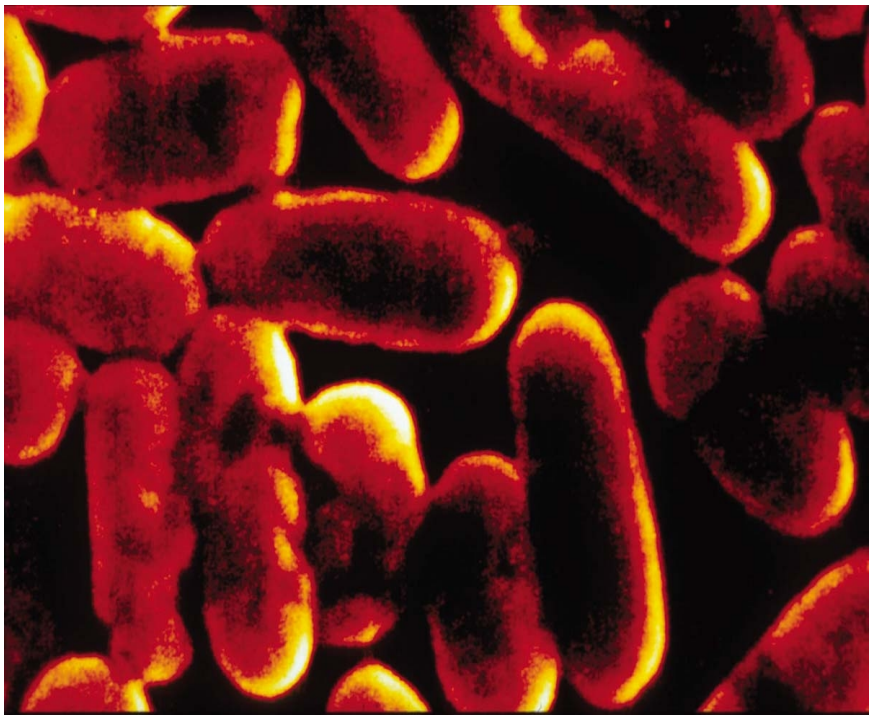
Final Report

CONTENTS

INTRODUCTION

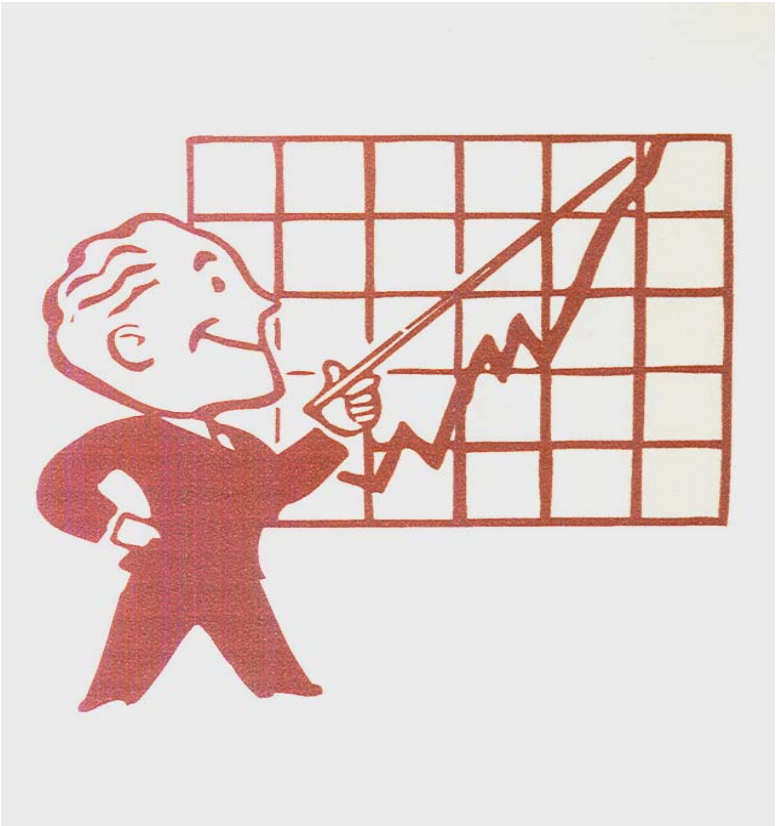


AIM AND OBJECTIVES



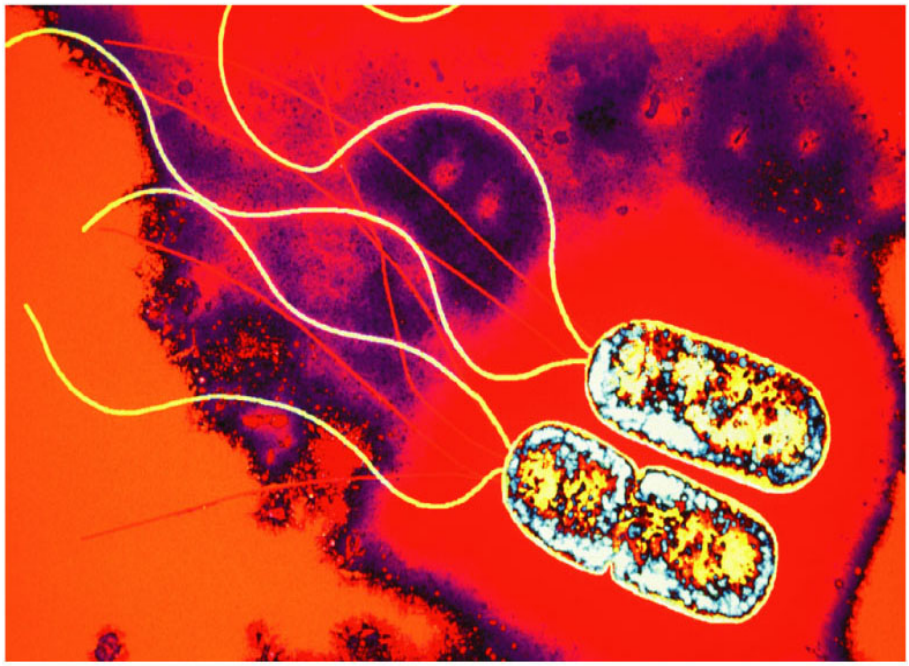
REVIEW OF LITERATURE

MATERIALS AND METHODS



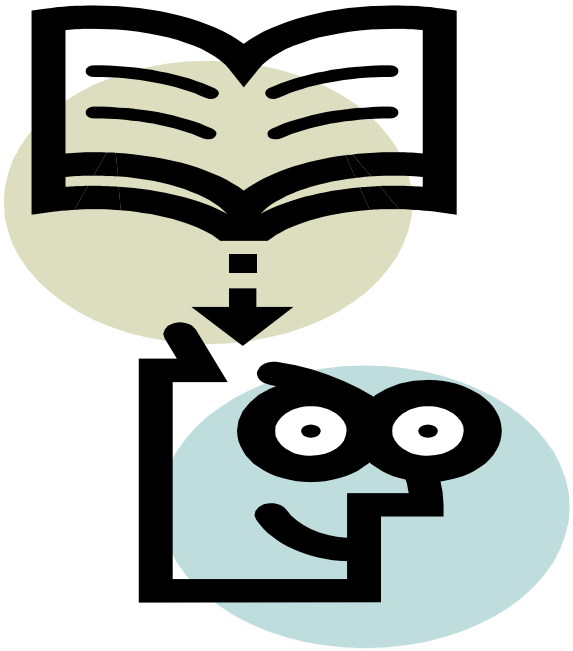
RESULTS

DISCUSSION



SUMMARY

CONCLUSION



BIBLIOGRAPHY


PROFORMA